alternative LOS structure Galα1 $\rightarrow$ 4Galβ1 $\rightarrow$ 4Glc (see Figure 1). Only the genetic basis of the 1291e mutant is now defined. It is a mutation of phosphoglucomutase (pgm), which precludes the synthesis of UDP-glucose, and hence the addition of the first residue of the lacto-N-neotetraose unit (Zhou et al., 1994, J. Biol. Chem. 269:11162; Sandlin and Stein, 1994, J. Bacteriol. 176:2930). It also has been shown that gale mutants of meningococcus or gonococcus produce truncated LOS in keeping with the inability to synthesize UDP-galactose (Robertson et al., 1993, Molec. Microbiol. 8:891; Jennings et al., 1993, Molec. Microbiol. 10:361). --

Please delete the paragraph at lines 13-23 on page 6 and insert the following paragraph in place thereof:

-- The present invention is directed to nucleic acids encoding glycosyltransferases, the proteins encoded thereby, and to methods for synthesizing oligosaccharides using the glycosyltransferases of the invention. Accordingly, in one aspect, the invention is directed to a purified nucleic acid that is hybridizable under moderately stringent conditions to a nucleic acid corresponding to the LOS locus of Neisseria, e.g., a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in (SEQ ID NO:1). Preferably, the nucleic acid of the invention is hybridizable to a portion of the coding sequence for a gene of the LOS locus, i.e., a portion of the nucleotide sequence shown in (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. --

Please delete the paragraph on page 6 line 24 to page 7 line 5 and insert the following paragraph in place thereof:

-- In specific embodiments, the invention relates to a nucleic acid that has a nucleotide sequence corresponding to or complementary to a portion of the nucleotide sequence shown in (SEQ ID NO:1) that encodes a functionally active glycosyltransferase. In a further aspect, the nucleic acid encodes a functionally active glycosyltransferase. In a specific embodiment, the invention is directed to a nucleic acid that has a nucleotide sequence corresponding to or complementary to the nucleotide sequence shown in (SEQ ID NO: 1). --

Please delete the paragraph at lines 15-17 on page 7 and insert the following paragraph in place thereof:

-- In specific embodiments, exemplified herein, the nucleic acid encodes a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8. --

Please delete the paragraph at lines 1-13 on page 8 and insert the following paragraph in place thereof:

-- In a primary aspect, the invention is directed to glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:12, SEQ ID NO:6, or SEQ ID NO:8 or a functionally active fragment thereof. The invention further contemplates a composition comprising a glycosyltransferase conjugated to a solid phase support, wherein the glycosyltransferase is selected from the group consisting of a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID NO:8, or a functionally active fragment thereof; a glycosyltransferase having an amino acid sequence of SEQ ID NO:4, or a functionally active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof; and a glycosyltransferase having an amino acid sequence of SEQ ID NO:6, or a functionally active fragment thereof. --

Please delete the paragraph from page 8 line 14 to page 9 line 3, and insert the following paragraph in place thereof:

-- Having provided novel glycosyltransferases, and genes encoding the same, the invention accordingly further provides methods for preparing oligosaccharides, *e.g.*, two or more saccharides. In specific embodiments, the invention relates to a method for adding GalNAc or GlcNAcβ1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ

ID NO:3 or SEQ ID NO:11; a method for adding Galβ1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:8; a method for adding Gal α1→4 to Gal, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:4; a method for adding GalNAc or GlcNAc β1→3 to Gal, comprising contacting a reaction mixture comprising an activated GalNAc or GlcNAc to an acceptor moiety comprising a Gal residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12; and a method for adding Gal β1→4 to GlcNAc or Glc, comprising contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a GlcNAc or Glc residue in the presence of the glycosyltransferase having an amino acid sequence of SEQ ID NO:6. --

Please delete the paragraph from lines 3-12 on page 11, and insert the following paragraph in place thereof:

-- Figure 2: Genetic map of the LOS locus based on the DNA sequence. Sequence information bp 1-2725 was obtained from plasmid pPstCla, bp 2725-5859 from plasmid p3400 (see materials and methods). IS refers to an area of the sequence that has homology to a previously reported neisserial insertion sequence IS1106 (Knight et al., 1992, Molec. Microbiol. 6:1565). The positions of the reading frames of *lgtA-E* are indicated. Three tracts of poly-G were found in *lgtA* (17 bp), *lgtC* (10 bp) and *lgtD* (11 bp) and are indicated by vertical black bars. --

Please delete the paragraph from lines 13-18 on page 11, and insert the following paragraph in place thereof:

-- Figure 3 (A and B): Homology of the protein products of *lgtA* (SEQ ID NO:11) and *lgtD* (SEQ ID NO:12). The primary structure of two proteins is very similar, particularly in the first half of the sequences. The glycine residues starting at position 86

reflect the coding of the poly-G regions in the respective genes. The Bestfit program of the GCG package was used and the symbols |, :, . represent degrees of similarity based on the Dayhoff PAM-250 matrix. --

Please delete the paragraph from lines 19-23 on page 11, and insert the following paragraph in place thereof:

-- Figure 4 (A and B): Homology of the protein products of *lgtB* (SEQ ID NO:8) and *lgtE* (SEQ ID NO:6). The primary 20 structure of two proteins is very similar, particularly in the first half of the sequences. These sequences also have significant homology to *lex-1* (Cope et al., i99i, Molec. Microbiol. 5:i113) or *lic2A* (High et al., i993, Molec. Microbiol. 9:1275) genes of *Haemophilus influenzae*. For meaning of symbols see Figure 3. --

Please delete the paragraph from page 11 line 24 to page 12 line 2, and insert the following paragraph in place thereof:

-- Figure 5 (A and B): Homology of the protein products of *rfaI* (SEQ ID NO:13) and *lgtC* (SEQ ID NO:4). The *E. coli rfal* and *rfaJ* genes are very closely related. They serve as glucosyl transferases of two glucose residues in the LPS core region (Pradel et al., 1992, J. Bacteriol. 174:4736). The glycines at position 54-56 in *lgtC* are encoded by the poly-G tract. For meaning of symbols see Figure 3. --

Please delete the paragraph from lines 11-29 on page 30, and insert the following paragraph in place thereof:

-- Accordingly, a method for preparing an oligosaccharide having the structure GalNAcβ1→3Ga1β1→4GlcNAcβ1→3Ga1β1→4Glc *(i.e.,* ganglioside) comprises sequentially performing the steps of:

a. contacting a reaction mixture comprising an activated Gal to an 15 acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

b. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof;

c. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a GlcNAcβ1→3Galβl→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID NO:8; and

d. contacting a reaction mixture comprising an activated GalNAc to the acceptor moiety comprising a Galβ1→4GlcNAcβ1→3Ga1β1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:12, or a functionally active fragment thereof. --

Please delete the paragraph from lines 1-14 on page 31, and insert the following paragraph in place thereof:

-- Similarly, a method for preparing an oligosaccharide having the structure Galβ1→4GlcNAcβ1→3Ga1β1→4Glc *(i.e.,* lacto-N-neotetraose) comprises sequentially performing the steps of:

a. contacting a reaction mixture comprising an activated Gal to an acceptor moiety comprising a Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO: 6, or a functionally active fragment thereof;

b. contacting a reaction mixture comprising an activated GlcNAc to the acceptor moiety comprising a Galβ1→4Glc residue in the presence of a glycosyltransferase having an amino acid sequence of SEQ ID NO:3 or SEQ ID NO:11, or a functionally active fragment thereof; and

c. contacting a reaction mixture comprising an activated Gal to the acceptor moiety comprising a G1cNAcβ1→3Ga1β1→4Glc residue in the presence of a glycosyltransferase having an amino acid of SEQ ID NO:8. --

Please delete the paragraph from lines 10-26 on page 38, and insert the following paragraph in place thereof:

-- A gene bank of *Neisseria gonorrhoeae* strain F62 genomic DNA was constructed by ligating ca 20 kb fragments obtained by incomplete digestion with *Sau3A* into *Bam*HI/*Eco*RI digested λ2001 (Karn et al., 1984, Gene 32:217). The phage library was screened by hybridization with random-primer-labeledplasmid pR10PI, and 5 clones were isolated by plaque purification. The phage from these clones were purified by sedimentation followed by flotation on CsCl (Davis et al., 1980, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), and the DNA was isolated. From one of these clones, two *Cla*I fragments of 4.9 and 3.4 kb were isolated by gel electrophoresis and recovery with Geneclean II (BIO 101 Inc., La Jolla, CA). These were ligated into *Cla*I cut pBluescript II SK- from Stratagene (La Jolla, CA) and called p4900 and p3400 respectively. p4900 contained a *Pst*I site in the insert and was subdivided into two clones containing inserts of 2.1 and 2.8 kb. The clone containing the 2.8 kb insert was called pPstCla. The inserts in p3400 and pPstCla were sequenced by the chain termination method (Sanger et al., 1977, Proc. Natl. Acad Sd. USA 74:5463) using Sequenase II, (United States Biochemical Co., Cleveland, OH). All of the sequence presented in SEQ ID NO:1 was completed in both directions. --

Please delete the paragraph from line 13 page 39 to line 3 page 40, and insert the following paragraph in place thereof:

-- Transformation of piliated *Neisseria gonorrhoeae* strain F62 was performed with plasmids isolated from *E. coil* (Klugman et al., 1989, Infect. Immun. 57:2066) and the transformants selected on GC agar (Swanson, 1978, Infect. Immun. 19:320) containing 2 μg/ml erythromycin. The fidelity of the genomic alteration of each of the gonococcal transformants was verified by sequencing the upstream and downstream junctions of the *ermC'* gene in their genomic DNA using a PCR technique. Two 5' biotinylated primers, GCCGAGAAAACTATTGGTGGA (SEQ. ID. NO:9) and AAAACATGCAGGAATTGACGAT) (SEQ. ID. NO:10), were synthesized; these were based on the *ermC'* sequence near its upstream and its downstream end respectively. The primers were designed such that their 3' ends pointed outward from the *ermC'* gene.

Each of these primers was used together with a suitable primer matching the sequence of the LOS locus near the putative insertion. PCR was performed according the instructions supplied with the GeneAmp PCR Reagent Kit from Perkin Elmer (Branchburg, NJ) using 25 cycles. In all instances the expected size product was obtained. The DNA sequence of these products was determined by purifying the PCR product on magnetic streptavidin beads from Dynal, Inc. (Lake Success, NY) and sequencing with the Sequenase II kit according to a protocol provided by Dynal, Inc., based on the method developed by Hultman et al (Hultman et al., 1989, Nucleic Acids Res. 17:4937). The sequences were analyzed by computer programs in the GCG package of Genetics Computer Group, Inc. (Madison, WI). --

Please delete the paragraph from lines 15-29 on page 41, and insert the following paragraph in place thereof:

-- A λ2001 bank of *Neisseria gonorrhoeae* strain F62 DNA was screened by hybridization with pR10PI and 5 clones were isolated. One of these clones, when digested with either *Cla*I or *Bfa*I and examined by Southern hybridization using pR10PI as the probe, gave rise to a pattern identical to that seen with genomic DNA. The appropriate *Cla*I fragments of this λ2001 clone were isolated and cloned into the *Cla*I site of pBluescript II SK-. The entire sequence of the 3400 *Cla*I fragment was determined. Mapping of the clone containing the 4900 bp *Cla*I fragment indicated that there was a single *Pst*I site in the clone about 2.8 kb from one side, allowing the clone to be divided into two subclones. Partial sequence of the ends of the 2.1 kb subclone indicated that it contained a coding frame homologous to the *E. coil* COOH-terminal portion of the α subunit of glycyl-tRNA synthetase *(glyS)* and the majority of the β subunit of this gene (Webster et al., 1983, J. Biol. Chem. 258:10637). The predicted length of DNA needed to match the *E. coli* sequence was present; this clone was not examined further. --

Please delete the paragraph from lines 1-9 on page 42, and insert the following paragraph in place thereof:

-- *DNA Sequence of the LOS Locus.* A summary of the features found by sequencing the two clones is illustrated in Figure 2. Following the *glyS* gene were foundfive closely spaced open reading frames. The last frame has 46 bp downstream of the termination codon a sequence typical of a rho independent termination signal. Subsequently, there is an area of ca 100 bp that has striking homology to the ISI1106 neisserial insertion sequence (Knight et al., 1992, Molec. Microbiol. 6:1565). Further elucidation of the nature of this locus, presented below, showed the five open reading frames code for LOS glycosyl transferases and hence they have been named *lgtA- lgtrE.* --

Please delete the paragraph from lines 10-29 on page 42, and insert the following paragraph in place thereof:

-- Searches for internal homology within this locus indicates that the DNA coding for the first two genes *(lgtA, lgtB)* is repeated as the fourth and fifth genes *(lgtD, lgtE)* and that interposed is an additional open reading frame, *lgtC.* This is in keeping with the data obtained by Southern hybridization presented above, in which pR10PI probe containing the *lgtB* and a small portion of the *lgtC* gene hybridized with two *Cla*I fragments, but with only one *Bfa*I fragment (see positions of the *Bfa*I sites in the LOS locus in Figure 2). In more detail, 16 bp following the stop codon of the tRNA synthetase *(glyS)* is the beginning of a stem loop structure followed closely by a consensus ribosome binding site (rbs), and within 6 bp is a TTG believed to be the initiation codon of *lgtA.* 2871 bp downstream from the beginning of the stem loop (closely following the stop codon of *lgtC)* there is an almost perfect repeat of the stem loop structure, the rbs, and the TTG initiation codon of *lgtD,* with the downstream sequence strongly homologous for about 500 bp. The sequences then diverge to some extent. However, at the beginning of *lgtB* and *lgtE* the homology again becomes nearly perfect for ca 200 bases to then diverge toward the latter part of the orfs. The similarity of the homologous proteins is illustrated in Figures 3 and 4. These comparisons, demonstrate the near-perfect conservation of the primary structure in the N-terminal portions of the molecules with increasing divergence toward the COOH-termini of the proteins. --